PB85-166767

Toxicity Reduction Test to Assist in Predicting Land Treatability of Hazardous Organic Wastes

(U.S.) Robert S. Kerr Environmental Research Lab., Ada, OK

Feb 85

U.S. Department of Commerce
National Technical Information Service
NTIS

PB85-166767

EPA/600/D-85/032
February 1985

A TOXICITY REDUCTION TEST SYSTEM TO ASSIST IN PREDICTING LAND TREATABILITY OF HAZARDOUS ORGANIC WASTES

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TECHNICAL REPORT DATA
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1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA/600/D-85/032		PB85 166767/AS

4. TITLE AND SUBTITLE	5. REPORT DATE
A Toxicity Reduction Test System to Assist in Predicting Land Treatability of Hazardous Organic Wastes	February 1985
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
J. E. Matthews and A. A. Bulich	RSKERL-Ada-8412
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
U.S. Environmental Protection Agency Robert S. Kerr Environmental Research Lab Post Office Box 1198 Ada, OK 74820	ABRD1A
	11. CONTRACT/GRANT NO.
	In-house
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
U.S. Environmental Protection Agency Robert S. Kerr Environmental Research Lab Post Office Box 1198 Ada, OK 74820	14. SPONSORING AGENCY CODE EPA-600/15

15. SUPPLEMENTARY NOTES

Project Officer: John E. Matthews

16. ABSTRACT

Migration of toxic organics contained in the water soluble fraction (WSF) of land applied hazardous wastes poses the most serious threat to ground-water resources. A toxicity reduction (TR) test system is being proposed that will serve to determine if, and to what extent, attenuation of WSF organic constituents will occur in a well-managed land treatment system. The test system employs reduction of acute toxicity exerted by WSF organics over time as the measurement criteria. Acute toxicity of the WSF for three loading rates is determined using a bacterial bioluminescence assay. Dose-response curves for each loading rate and subsequent time interval are compared with those of previous sample sets. The waste is predicted to be a candidate for land treatment if a significant reduction of acute toxicity exists during the experimental period at any of the loading rates tested. The maximum acceptable initial loading rate (MAIL) for waste application is predicted from these data. The predicted MAIL rate is the highest rate tested in which toxicity reduction for the waste-soil WSF progresses steadily during the experimental period with the Day-42 EC50 approaching 100 percent. The most extensive use of the procedure to date has involved evaluation of land treatability for oily wastes from different sources within a refinery; i.e., lagoon bottom sludge, API separator sludge, slop oil emulsion solids, and Dissolved Air Flotation unit skimmings. Results from these evaluation tests indicate the potential usefulness of the TR procedure as one of the initial tests conducted in a laboratory screening test sequence for predicting land treatability potential of organic hazardous wastes.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Hazardous waste Toxicity Bioassay Land treatment	Microbial assay Treatability Screening test Oily waste	68C

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release to Public	Unclassified	30
	20. SECURITY CLASS *(This page)*	22. PRICE
	Unclassified	

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

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A TOXICITY REDUCTION TEST SYSTEM TO ASSIST IN PREDICTING LAND TREATABILITY OF HAZARDOUS ORGANIC WASTES

by

J. E. Matthews[1] and A. A. Bulich[2]

ABSTRACT: Migration of toxic organics contained in the water soluble fraction (WSF) of land applied hazardous wastes poses the most serious threat to groundwater resources. Wastes containing toxic organics which are potentially leachable must be applied to the land at a rate which will allow attenuation of such constituents before their migration from the treatment zone can occur.

A toxicity reduction (TR) test system is being proposed that will serve to determine if, and to what extent, attenuation of WSF organic constituents, including both parent compounds and intermediate transformation products, will occur in a well-managed land treatment system.

The test system employs reduction of acute toxicity exerted by WSF organics over time as the measurement criteria. Four sample sets of selected waste-soil combinations are prepared in duplicate for extracting with deionized water (DW) at 14-day intervals during a 42-day experimental period. Acute toxicity of each DW extract is determined using a bacterial bioluminescence assay. Dose-response curves for each loading rate and subsequent time interval are compared with those of previous sample sets. The waste is predicted to be a candidate for land

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treatment if a significant reduction of acute toxicity exists during the experimental period at any of the loading rates tested. The maximum acceptable initial loading rate (MAIL) for waste application is predicted from these data. The predicted MAIL rate is the highest rate tested in which toxicity reduction for the waste-soil DW extract progresses steadily during the experimental period with the Day-42 EC50 approaching or exceeding 100 percent. The EC50 is defined as the effective concentration of the DW extract which causes a 50 percent decrease in bacterial light output. The higher the EC50 the lower the toxic effect; therefore, a 42-day EC50 approaching 100 percent signifies toxicity reduction in a waste-soil DW extract.

The TR procedure currently is being used by the EPA's Hazardous Waste Land Treatment Research Program to assist in evaluating land treatability potential for a variety of organic hazardous wastes. The most extensive use of the procedure to date has involved evaluation of land treatability for oily wastes from different sources within a refinery, i.e., lagoon bottom sludge, API separator sludge, slop oil emulsion solids, and Dissolved Air Flotation unit skimmings. Additional evaluation tests have been conducted using wastes from the wood preserving and paint industries. Results from these evaluation tests indicate the potential usefulness of the TR procedure as one of the initial tests conducted in a laboratory screening test sequence for predicting land treatability potential of organic hazardous wastes.

Although the research described in this article has been funded wholly or in part by the US EPA, it has not been subjected to the Agency's peer and administrative review and therefore may not necessarily reflect the views of the Agency and no official endorsement should be inferred.

KEY WORDS: toxicity, tests, bioassay, treatability, land treatment, hazardous waste, screening.

Regulations governing treatment of hazardous waste in land treatment units [1][3] stipulate that hazardous constituents contained in the applied waste must be detoxified or otherwise rendered innocuous within the defined soil treatment zone. A demonstration of treatment is required to obtain a HWLT facility operating permit. This demonstration must focus on maximizing attenuation of hazardous constituents via natural soil processes and interactions and minimizing their escape to ground water, surface water and air. It is recommended that the treatability studies address, at a minimum, degradation/transformation, mobility and toxicity of hazardous constituents [2].

One promising approach to generating the diverse data needed to make initial land treatability decisions is through the use of a standardized battery of relatively rapid laboratory screening tests, each of which addresses at least one of the aforementioned areas of concern. Following an initial waste and soil characterization phase, this approach involves subjecting the waste in question to a prescribed sequence of screening procedures in which the proposed site soil is used as the treatment medium. Integrated data from the different areas of testing are used to categorize land treatability potential of a specific waste if applied to a specific soil. The major objective of laboratory screening is to identify any treatability problem rapidly and at a minimum of cost. Bench and/or pilot-scale verification studies are conducted to complete the land treatment demonstration. This phased approach to conducting a land treatment demonstration program is depicted in figure 1.

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[3]The numbers in brackets refer to the list of references appended to this paper.

Screening tests which require extraction and analyses for specific chemical constituents also require the largest resource allocation. Therefore, the phase II laboratory screening sequence should begin with procedures which utilize gross parameters relative to the total pollutant matrix as the measurement criteria. Results from this first tier of screening tests can be used to make decisions pertaining to which subsequent screening tests should be conducted and to define the highest waste loading rate to be used in these tests.

A major concern associated with land treatment of hazardous wastes is adequate protection of ground-water resources. The water soluble fraction (WSF) of land-applied wastes possesses the greatest potential for leaching and subsequent ground-water contamination [3,4]. Any waste which contains organic hazardous constituents in the WSF must be applied at a rate which will allow for detoxification of such constituents within the treatment zone before leaching into the unsaturated zone can occur. Therefore, one of the first tests conducted in the screening sequence should address leaching and detoxification potentials of these WSF organic constituents.

This paper presents a toxicity reduction (TR) test system which can be utilized as one of the initial screening tests conducted during the phase II land treatability laboratory test sequence [figure 2]. Results can be used to predict if, and to what extent, detoxification of the WSF organic pollutant matrix will occur in the site soil. Coupled with results from microbial activity assays, comparisons of detoxification of the WSF of several waste-soil combinations allows definition of the maximum waste loading rate which should be used in subsequent screening tests.

EXPERIMENTAL METHOD

If the WSF of any hazardous waste exhibits a significant level of acute toxicity utilizing a specific bioassay system, then reduction of the toxicity as measured by that bioassay becomes a measure of treatability. The basic design concept of the proposed test system assumes that for a given waste-soil loading rate, significant toxicity reduction of the waste-soil WSF pollutant matrix during the experimental period defines a positive land treatability potential at that loading rate.

The relative acute toxicity of the WSF is determined by conducting a bacterial bioluminescence assay (designated as Microtox)[4] on the deionized water (DW) extract of the waste and specific waste-soil combinations. The Microtox system utilizes a suspension of marine luminescent bacteria (Photobacterium phosphoreum) as bioassay organisms. Suspensions with approximately 10^6 bioluminescent organisms in each are "challenged" by addition of serial dilutions of the DW extract. A reduction of light output reflects physiological inhibition thereby signifying presence of toxic constituents in the sample.

This assay was selected for use because of the small volume of sample required, the ease of determining the end point, and its reported effectiveness in determining relative acute toxicity of complex wastewaters containing toxic organic constituents [5,6]. A recent report published by the Canadian Petroleum Association [7] concluded that the bacterial bioluminescence assay

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[4]Microtox is a trademark of Beckman Instruments, Inc., Carlsbad, CA. Reference to equipment brand names or supplies in this paper is not to be interpreted as an endorsement of that product or supplies by the U. S. Environmental Protection Agency or the American Society for Testing and Materials.

is a viable method of screening for apparent toxicity in complex waste drilling fluids. Results correlated closely with those from rainbow trout bioassays. The report also recommends that this assay be utilized as a tool in evaluating the effects of these drilling fluids on soils.

Acute toxicity reduction of WSF organics as depicted by bacterial bioluminescence assays is used as the measurement criteria for predicting land treatability potential. The effective concentration of the DW extract which causes a 50 percent decrease in light output under the conditions of the test (EC50) is used as the primary unit for comparing toxicity reduction.

A TR experiment requires preparation of 32 test units. Each unit consists of a 500 ml flask containing 50 g of site soil which has been crushed and sieved to 2 mm. Three appropriate weights/volumes of waste are thoroughly mixed with the soil to achieve the desired loading rates. A routine experiment employs 4 sample sets of test units. Each set consists of 8 units including duplicates for 3 loading rates and a control. Results of preliminary toxicity tests run on extracts of the waste, soil, and 4 waste-soil loadings (1, 5, 10, and 15 wt.%) are used to select loading rates for a toxicity reduction experiment. Loading rates generally are selected in an arithmetic or geometric series that will allow the Day 0 EC50 to fall within a range of 20-60%. Sample sets are sacrificed for DW extraction and toxicity testing on Day 0 and at 14-day intervals during a routine 42-day experiment.

Test units are maintained at room temperature (22 $\pm$ 2 C). Soil moisture is maintained between 40 percent and 70 percent of the soil moisture holding capacity (MHC). These values are well within the 30-90 percent range presented by Dibble and Bartha [8] as being favorable for biodegradation

of oil sludge in soil. When the soil moisture reaches 40 percent of its MHC, the waste-soil combination is mixed and DW added to return the moisture level to 70 percent.

The extraction procedure consists of adding 400 ml of DW to each test unit at the specified time interval, tightly sealing the unit, and mixing for 22 $\pm$ 2 hours in a rotary tumbler mixer at approximately 30 rpm. This procedure provides for sufficient agitation on the mixture so that sample surfaces are continually brought into contact with the extracting medium. Following this mixing period, the resulting slurry for each test unit is filtered under pressure through 0.45 micron filter paper. Osmotic adjustment of the resulting filtrate is then made because the test organism is a marine species.

Bacterial bioluminescence assays are conducted using serial dilutions of each osmotically adjusted filtrate. Dose-response curves, EC50 values, and 95 percent confidence limits are calculated for each loading rate at each time interval using data reduction procedures described in the manufacturer's operating manual [9] or other appropriate methods for reducing acute toxicity data [10]. The extent of toxicity reduction is determined by comparing subsequent EC50 values for a given loading rate with those for the Day 0 sample. Control soil samples are extracted and tested to determine if the soil matrix itself exhibits a residual toxicity.

Experimental results are used to predict the maximum acceptable initial loading (MAIL) rate for applying the tested waste to the tested soil. The MAIL rate is the highest at which acute toxicity reduction for WSF organics contained in the DW extract progresses steadily during the experimental

period. Since the higher the EC50 the lower the toxicity, if the EC50 for Day 42 approaches or exceeds 100 percent significant toxicity reduction in a waste-soil extract is indiciated. The calculated EC50 can exceed 100 percent if the undiluted (100 percent dilution) DW extract does not elicit a 50 percent decrease in light output from the luminescent bacteria. This is a typical response for many control soils. The highest loading rate (HLR) which should be considered in subsequent screening tests is considered to be twice the MAIL rate.

A loading rate is not considered to be acceptable for initial loading if the Day 14 EC50 for that rate does not reflect an initiation of toxicity reduction regardless of what happens in the subsequent sample sets. Higher EC50 values reflect lower toxicity; therefore, an initiation of toxicity reduction is indicated if the Day 14 EC50 is significantly higher than the Day 0 EC50. A significant increase exists when 95 percent confidence intervals for the EC50 values of the Day 0 and Day 14 sample sets do not overlap. The basis for this rejection criteria is the high potential for migration of toxic water soluble organics below the treatment zone during such a long lag period. Significant toxicity reduction in subsequent sample sets, however, might indicate the potential for the use of this higher loading rate once microbial acclimation to the waste has occurred.

A loading rate at which the EC50 of the Day 28 sample decreases significantly from the Day 14 level may also be unacceptable for initial loading. Such a condition may reflect the formation of a significant quantity of toxic intermediates which are soluble and/or increased solubility of toxic parent compounds.

Under the test conditions, the former is more likely to occur. The potential exists for migration of these toxics below the treatment zone if a subsequent lag period in their toxicity reduction occurs. This possibility is being investigated in on-going land treatability studies.

Land treatability potential for a variety of hazardous wastes currently is being evaluated at the Robert S. Kerr Environmental Research Laboratory (RSKERL) as part of EPA's Hazardous Waste Land Treatment Research Program. Treatability predictions for selected organic wastes are made based on results from a prescribed battery of laboratory screening tests which address all potential areas of concerns: degradation, migration and toxicity. The TR procedure is included in this screening test battery. Bench-scale and/or field-plot studies are then used to verify these predictions.

EXPERIMENTAL RESULTS AND DISCUSSION

The TR procedure described is the first test conducted in the laboratory screening test sequence (figure 2). Results are used to predict an acceptable initial loading rate and to define the highest rate to be used in subsequent screening tests. Wastes for which TR experiments have been completed are presented in table 1.

TR experimental results indicate that each of these wastes is a potential candidate for land treatment; however, environmentally acceptable loading rates predicted vary considerably. Predicted acceptable loading rates for each waste type are presented in table 2.

Results from TR experiments for the lagoon bottom sludge compared favorably with those generated during subsequent field-plot experiments. The EC50 profiles for the lagoon bottom sludge TR and field-plot experiments are presented in figures 3 and 4 respectively.

Laboratory TR results indicated that 6 waste wt.% would be an environmentally acceptable loading rate for initial application of the lagoon bottom sludge to the loam soil used in this experiment. Toxicity reduction at this loading rate progressed steadily during the experiment. There also was no indication that a significant quantity of toxic intermediates would be formed if this loading rate were used or that solubility of toxic parent compounds would be increased.

Soil-core samples from field-plots to which the lagoon bottom sludge was applied were subjected to DW extraction and toxicity testing in the same manner as laboratory TR experimental units. EC50 profiles for these samples again indicated that 6 waste wt.% would be an environmentally acceptable loading rate for initial application of this waste to the loam soil. The increased toxicity observed 10 weeks following waste reapplication most probably reflected the extended period of hot, dry weather which occurred in late summer. Since supplemental moisture was not added during that period, microbial activity would have decreased; and further transformation of any toxic intermediates formed would have been minimized. In addition, solubility of some toxic parent compounds could have increased under these conditions because in general, solubility increases with increasing temperature. After cessation of the hot, dry conditions, toxicity reduction appeared to progress in a normal fashion.

Results from a TR experiment using the lagoon bottom sludge and another soil type (silty loam) gave essentially the same results as those using the loam soil. Six (6) waste wt.% was indicated to be an environmentally acceptable loading rate for initial application to the silty loam soil.

Results from the TR experiment for the API sludge were used to compare with results generated during an extensive field-plot research project conducted by the University of Oklahoma [11]. The EC50 profiles for this experiment are presented in figure 5. The 4 wt.% MAIL rate predicted from the TR results agreed favorably with the field-plot data.

TR experiments for the remaining 4 wastes listed in table 1 have been conducted as part of a hazardous waste land treatability screening program currently under evaluation at the RSKERL. The EC50 profiles for these 4 wastes are presented in figures 6-9.

Comparable oil contents for the 4 oily wastes tested were 25, 51, 15 and 11 wt.% respectively for the lagoon bottom, API separator, slop oil and DAF samples. Predicted MAIL rates for each waste are similar when compared on a waste weight percent basis (6, 4, 5 and 6 waste wt.%). When converted to an oil in soil content basis, however, there is a significant difference in predicted MAIL rates for the lagoon bottom and API samples (1.5 and 2.0 oil wt.%) when compared to those for the slop oil and DAF samples (0.75 and 0.65 oil wt.%).

The current practice at many operating oily waste land treatment facilities is to use oil in soil content as the primary loading parameter. Variations in predicted MAIL rates for the 4 oily wastes indicate that there is not a universal oil in soil content that will be environmentally acceptable for initial loading at every land treatment facility. The oil in soil content acceptable for initial loading is both soil and waste specific with the waste WSF pollutant matrix being the most dominant factor of the two. This lends credence to the current requirement that land treatability studies be conducted using the waste to be treated and the soil to which that waste will be applied.

Two (2) TR experiments have been conducted for the wood preserving treatment sludge. Each of the 3 loading rates used in the first experiment was considered to be environmentally unacceptable (figure 8). Although the Day 0 EC50 was noticeably higher at the lowest loading rate tested (0.4 waste wt.%), there was no apparent toxicity reduction between any of the sample sets at any of the 3 rates tested. A second experiment was conducted using loading rates of 0.1, 0.2 and 0.3 wt.% to try to predict a MAIL rate which would be compatible with the environment. The predicted MAIL rate for this waste was the lowest of the 6 rates tested, 0.1 waste wt.%.

The most significant toxic organic identified in the wood preserving waste was pentachlorophenol (PCP). The concentration of PCP was much higher than any of the other potential toxicants. The TR experimental data indicate that wood preserving wastes containing PCP can be land treated in an environmentally acceptable manner; however, such a practice will require careful management to control the PCP content in the soil. There appears to be a sharp demarcation between an initial PCP loading rate which will be environmentally acceptable and one which will be unacceptable.

The paint waste exhibited the most atypical toxicity reduction pattern of those tested to date. Day 0 samples for each of the experimental loading rates exhibited a high WSF acute toxicity level; however, Day 14 samples reflected at least a 6-fold decrease in this toxicity. The Day 14 EC50 for the WSF of the low loading rate sample (4 waste wt.%) exceeded 100 percent. This indicates the presence of a high quantity of toxic volatile organics in the WSF of the paint waste. The Day 14 EC50 values for the 8 and 12 waste wt.% loading rates, however, indicate that a significant quantity of semi-volatile toxic organics

also are contained in this waste. Since none of the volatile constituents identified in this waste appear on the list of hazardous constituents presented in Appendix VIII to Part 261, Section 3001, Subpart D of the Resource Conservation and Recovery Act, this volatilization does not preclude the use of land treatment as a management alternative. Air-stripping of volatile components, however, should be considered in the final determination of an acceptable waste loading rate.

The EC50 profiles for each of the 6 wastes tested to date have exhibited the same general trend. Acute toxicity, as defined by the bacterial bioluminescence assay, increased with increased loading rate within a given sample set at any point in time and decreased for a given loading rate with each subsequent sample set as a function of time. In addition, as the loading rate increased for a given waste, significant lag periods and/or reversals in toxicity reduction were common occurrences as indicated by comparisons of EC50 95 percent confidence intervals.

Initial sharp reduction in toxicity as depicted in some EC50 profiles indicate that volatilization of toxic organics may have contributed to toxicity reduction during these experiments. In such cases, the major impact most likely occurred within a short time following mixing of the waste and soil. A simple experiment was conducted to check for volatilization potential of WSF toxic organics. Fifty grams of an oily sludge were added to three flasks and extracted with DW on Days 0, 14 and 28. EC50 values for the DW extracts were 18.0, 24.0 and 24.5 respectively indicating the potential for loss of volatile toxicants via air-stripping following waste applications to soil.

The significance of volatilization as a factor in toxicity reduction is dependent on both the organic pollutant matrix and the waste loading rate. Of the wastes tested to date, volatilization of toxic organics was most apparent in the paint waste TR experiment (figure 9). In contrast to results of previous experiments, sharp reductions in toxicity during the 14 days following waste application were exhibited at each of the three loading rates tested.

SUMMARY

A toxicity reduction procedure employing a bacterial bioluminescence assay currently is being used as one of the initial tests conducted in a laboratory screening test battery for determining land treatability potential of hazardous waste. Experimental results obtained thus far demonstrate that the TR procedure can be used to determine the detoxification potential of WSF organics which provide the greatest threat to ground-water resources. This allows prediction of an environmentally acceptable loading rate for the waste as well as definition of the highest loading rate which should be used in subsequent screening tests.

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Table 1. Hazardous Waste Types Subjected to TR Experiments.

Waste Types	Soil Type		
	Loam	Silty Loam	Clay Loam
1. Oily Lagoon Bottom Sludge	X	X	
2. API Separator Bottoms		X	
3. Slop Oil Emulsion Solids			X
4. DAF Skimmings			X
5. Wood Preserving Treatment Sludge			X
6. Paint Waste			X

Table 2. Predicted Loading Rates for Hazardous Wastes Subjected to TR Experiments.

Waste Type	Predicted Loading Rate (Waste wt.%)[a]	
	MAIL[b]	HLR[c]
1. Oil Lagoon Bottom Sludge	6	12
2. API Separator Sludge	4	8
3. Slop Oil Emulsion Solids	5	10
4. DAF Skimmings	6	12
5. Wood Preserving Treatment Sludge	0.1	0.2
6. Paint Waste	4	8

a. Grams of waste applied and incorporated in 100g of dry soil.

b. Maximum acceptable initial loading rate predicted for applicaiton of tested waste in tested soil.

c. Highest loading rate which should be considered for use in subsequent treatability testing.

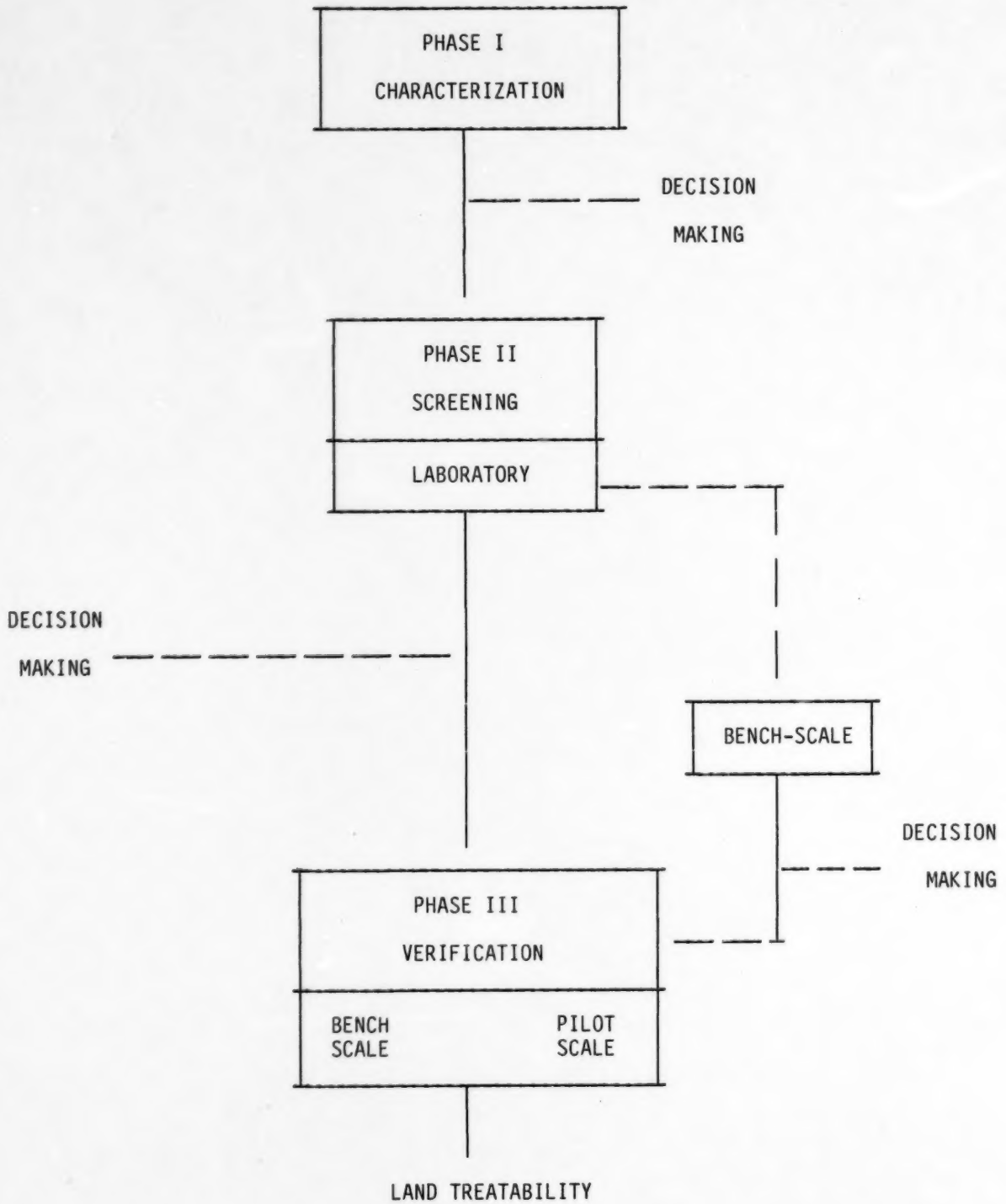

Figure 1. Flow Diagram for Land Treatability Demonstration

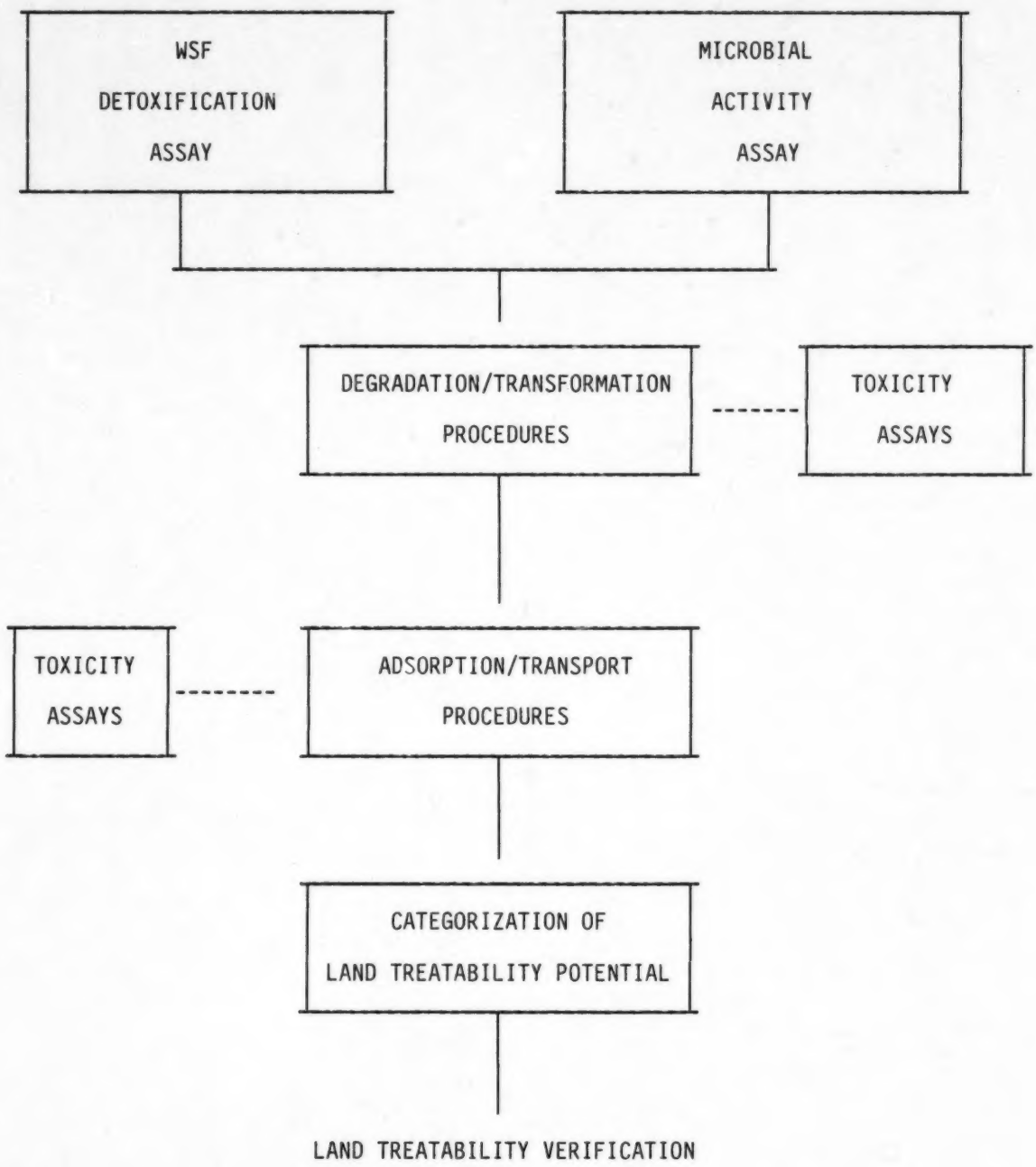

Figure 2. Laboratory Screening Test Sequence for Predicting Land Treatability Potential of Hazardous Waste.

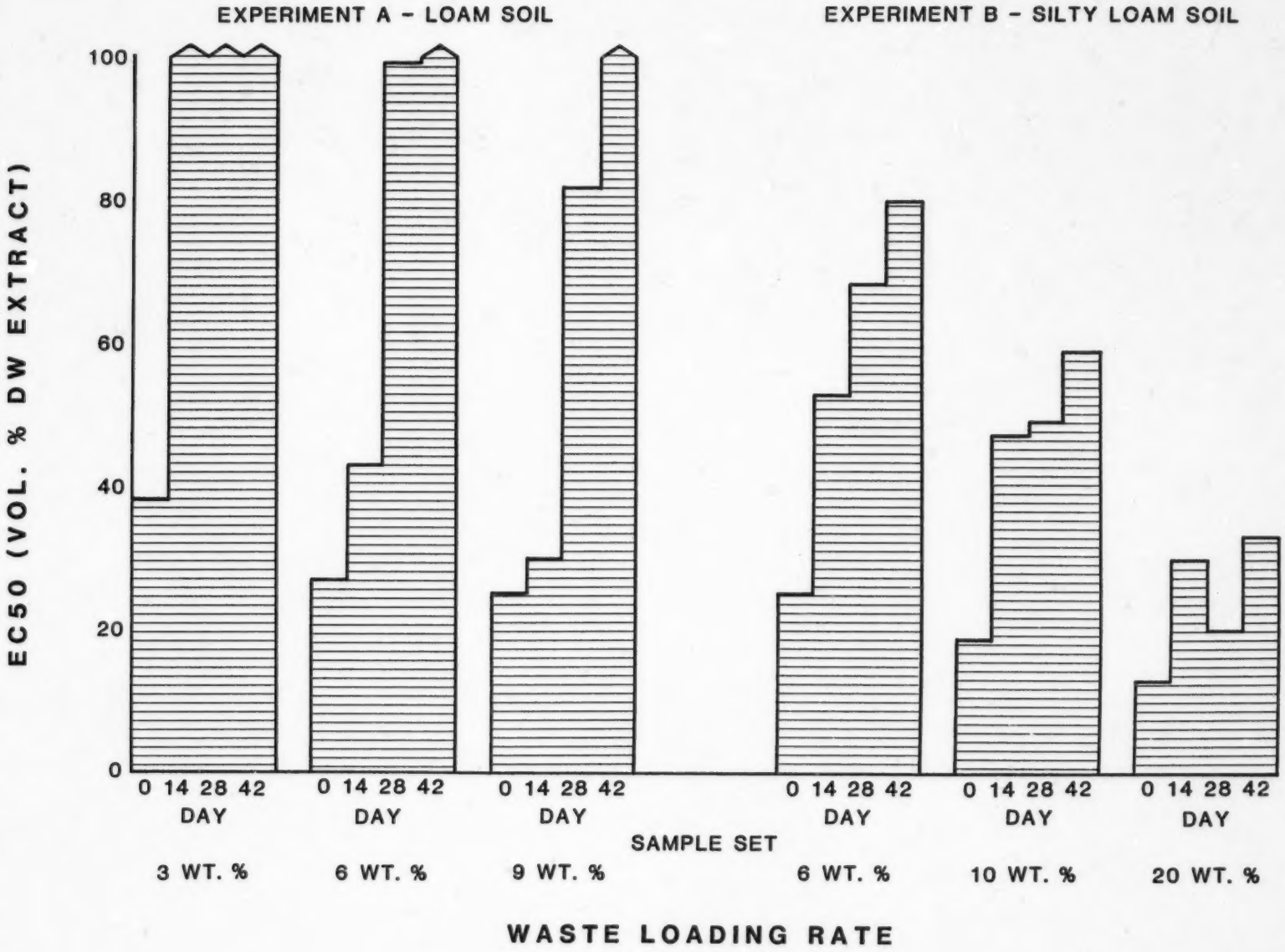

Figure 3. EC50 Profiles for Lagoon Bottom Sludge TR Experiments

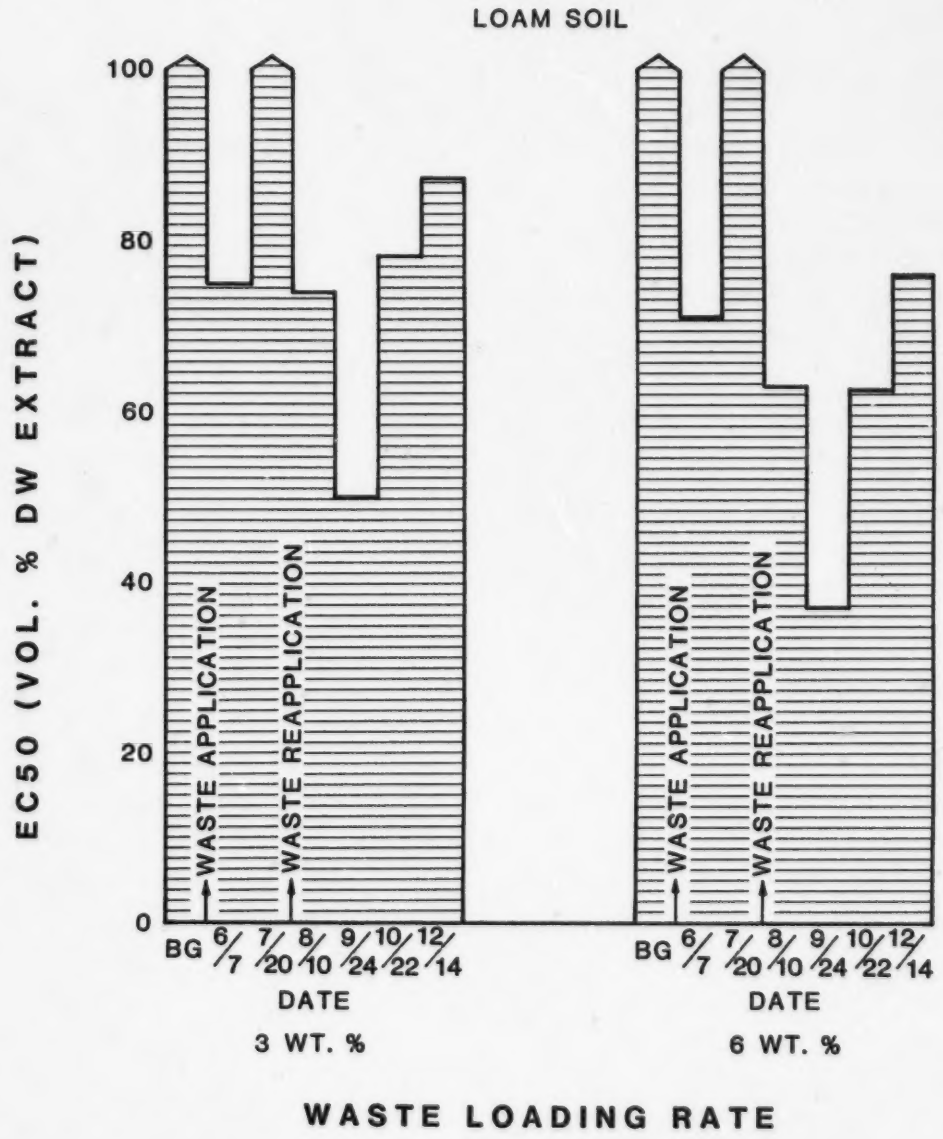

Figure 4. EC50 Profiles for Lagoon Bottom Sludge Field-Plot Core Samples

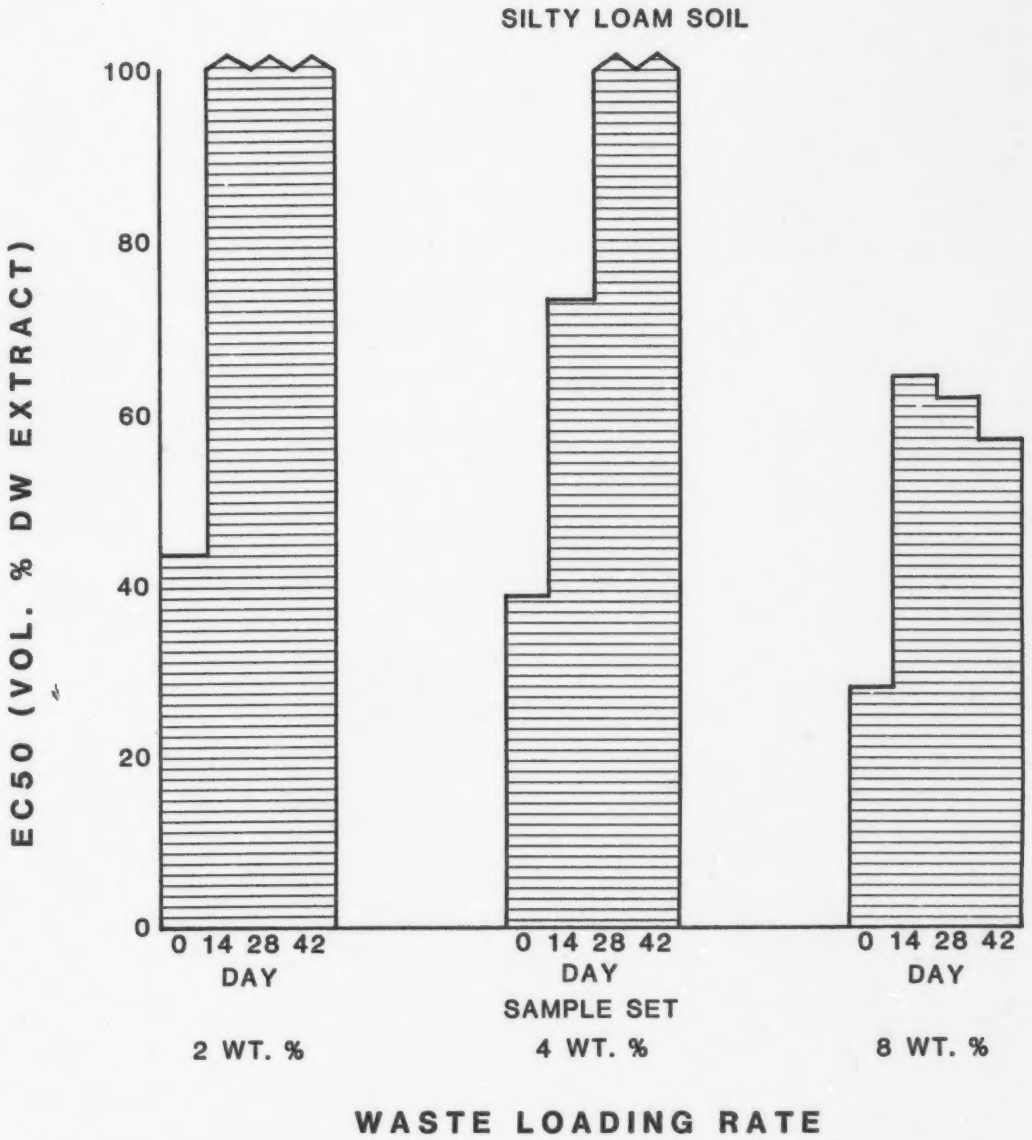

Figure 5. EC50 Profiles for API Separator Bottoms TR Experiment

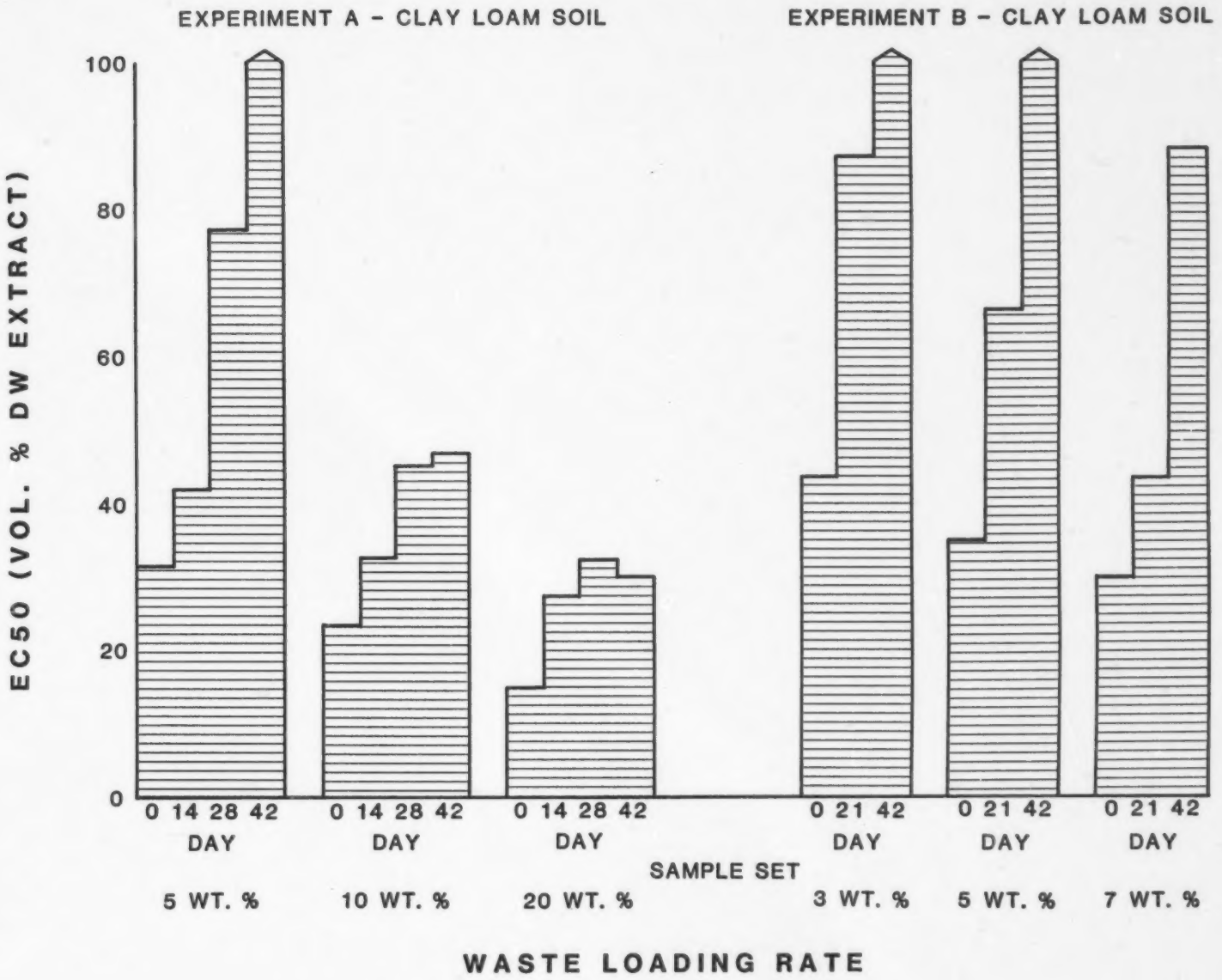

Figure 6. EC50 Profiles for Slop Oil Emulsion Solids TR Experiment

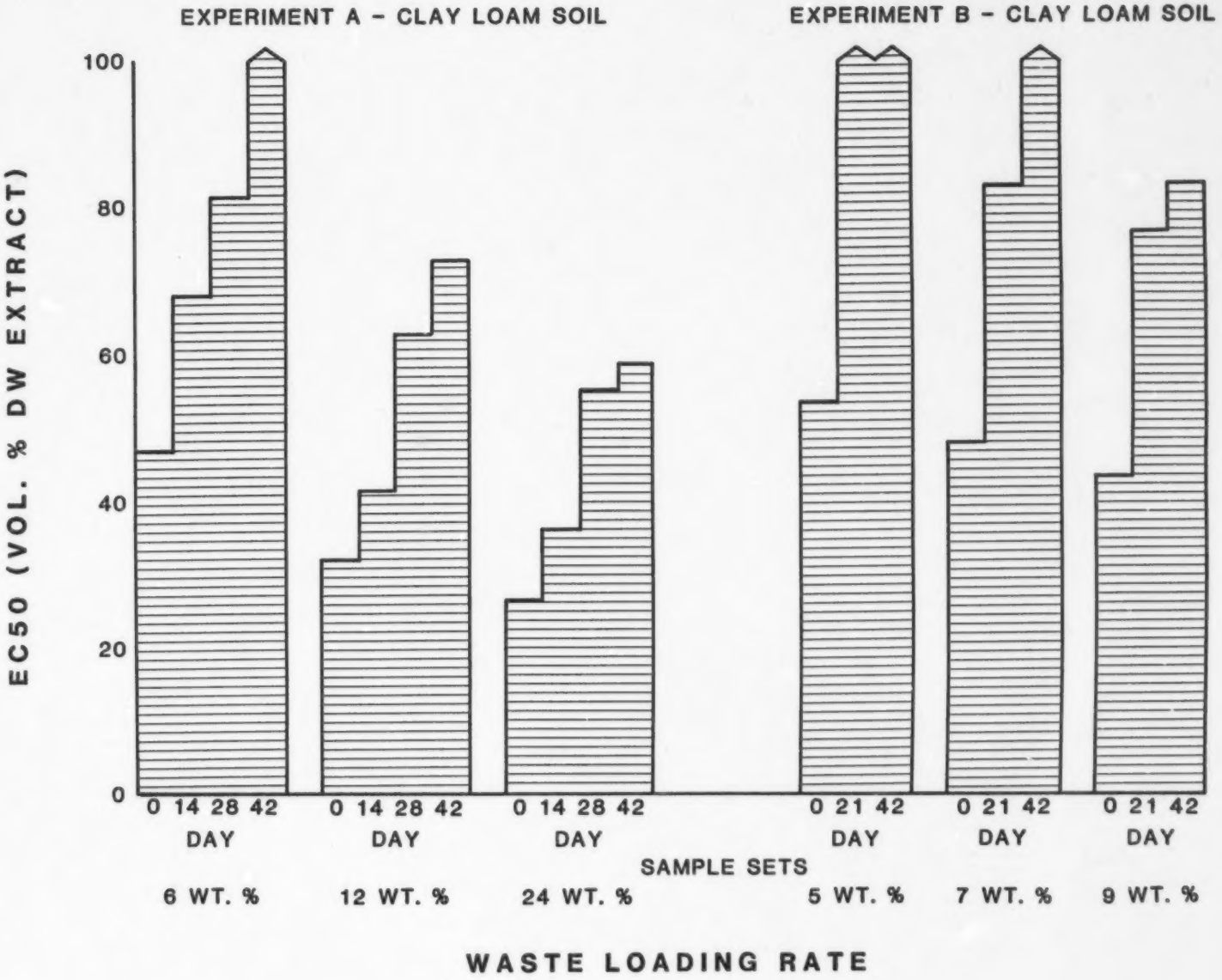

Figure 7. EC50 Profiles for DAF Skimmings TR Experiment

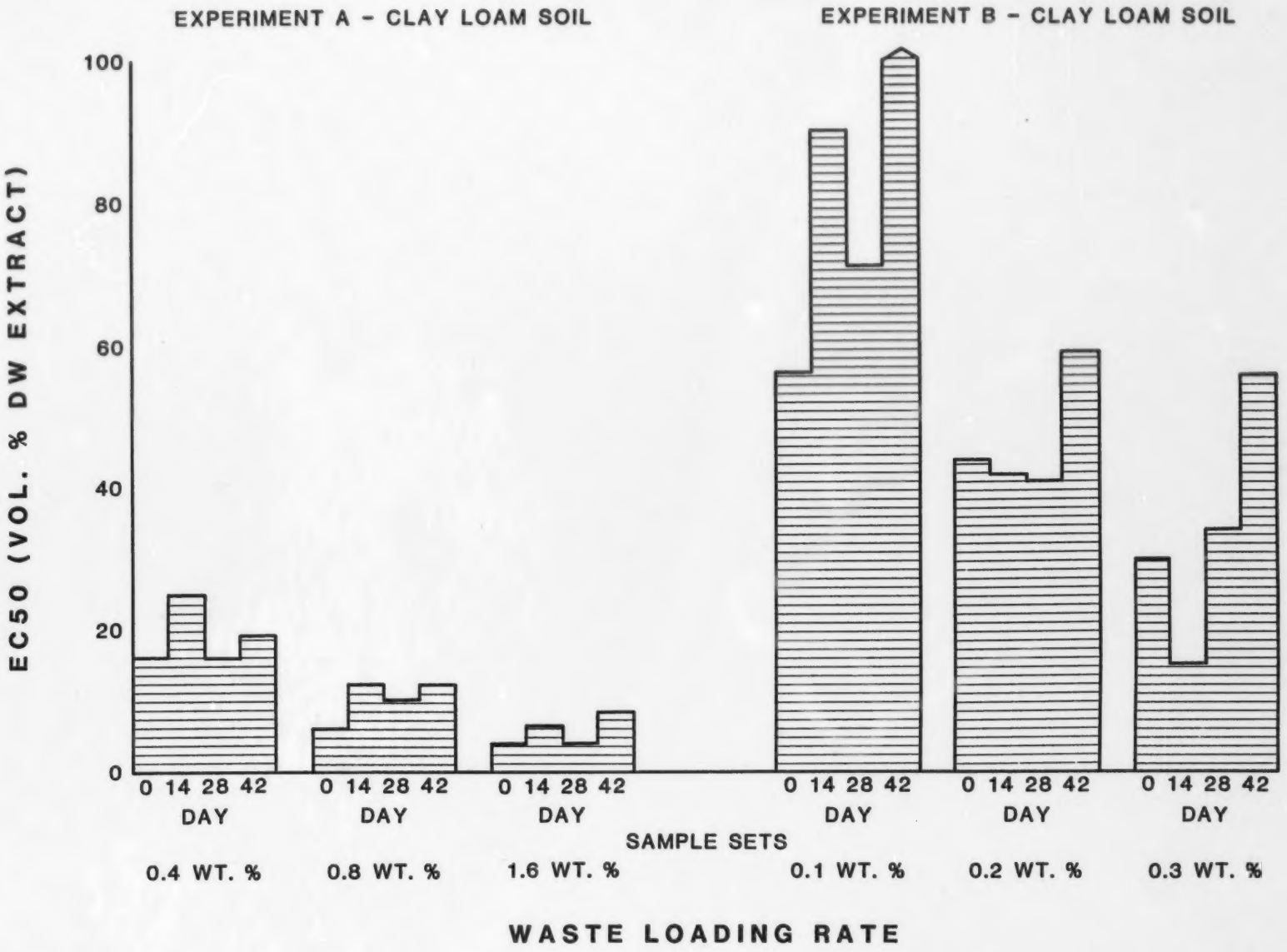

Figure 8. EC50 Profiles for Wood Preserving Treatment Sludge TR Experiment

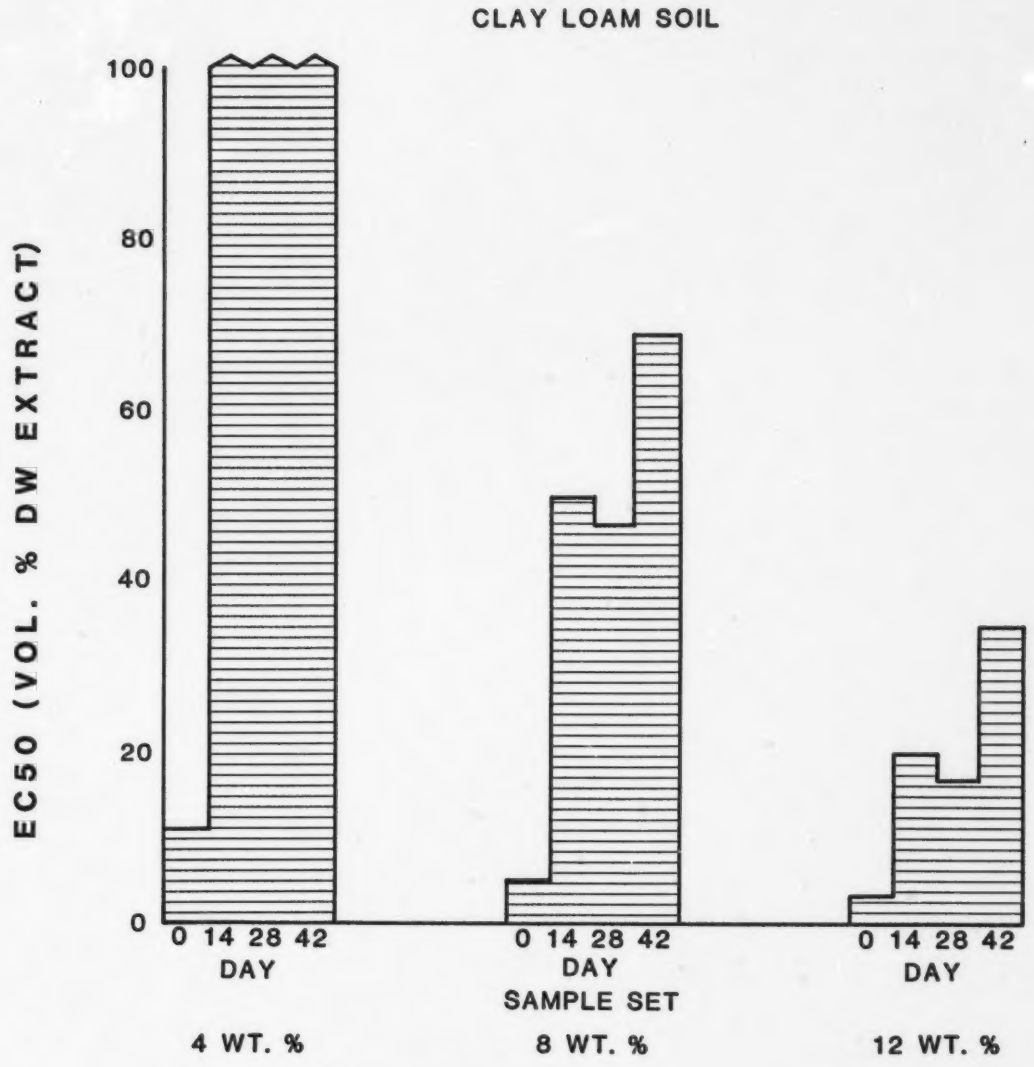

Figure 9. EC50 Profiles for Paint Waste TR Experiment